



Application No. 00/603,663

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

The claims have been amended as follows:

1. (Amended) A method for selecting tester proteins capable of binding to a target peptide or protein, the method comprising:

expressing a library of tester fusion proteins in yeast cells, each tester fusion protein [being a fusion protein comprised of] comprising either an activation domain or a DNA binding domain of a transcription activator and a tester protein having a diversity of at least 1×10^7 within the library, the tester protein comprising a first polypeptide subunit whose sequence varies within the library, a second polypeptide subunit whose sequence varies within the library independently of the first polypeptide, and a linker peptide which links the first and second polypeptide subunits;

expressing a target fusion protein in the yeast cells expressing the tester fusion proteins, the target fusion protein comprising either the DNA binding domain or the activation domain of the transcription activator which is not comprised in the tester fusion proteins, and a target peptide or protein; and

selecting those yeast cells in which a reporter gene is expressed, the expression of the reporter gene being activated by a reconstituted transcriptional activator formed by binding of the tester fusion protein to the target fusion protein.

2. (Amended) The method of claim 1, wherein expressing the library of tester fusion proteins includes transforming a library of tester expression vectors into the yeast cells which contain a reporter construct comprising the reporter gene whose expression is under transcriptional control of [a] the reconstituted transcription activator [comprising an activation domain and a DNA binding domain], each tester expression vector comprising

a first transcription sequence encoding either the activation domain or the DNA binding domain of the transcription activator, and

[a first nucleotide sequence encoding the first polypeptide subunit,

a second nucleotide sequence encoding the second polypeptide subunit, and

a linker sequence encoding a linker peptide that links the first nucleotide sequence and the second nucleotide sequence polypeptide subunits]

a sequence encoding one of the tester proteins.

B

4. (Amended) The method of claim 1, wherein the steps of expressing the library of tester fusion proteins and expressing the target fusion protein include causing mating between first and second populations of haploid yeast cells of opposite mating types,

wherein

the first population of haploid yeast cells comprises

a library of tester expression vectors for the library of tester fusion proteins, each tester expression vector comprising

a first transcription sequence encoding either the activation domain or the DNA binding domain of the transcription activator, and

[a first nucleotide sequence encoding the first polypeptide subunit,

a second nucleotide sequence encoding the second polypeptide subunit,

and

a linker sequence encoding a linker peptide that links the first nucleotide sequence and the second nucleotide sequence polypeptide subunits]

a sequence encoding one of the tester proteins;

the second population of haploid yeast cells comprises a target expression vector comprising

a second transcription sequence encoding either the activation domain or the DNA binding domain of the transcription activator which is not expressed by the library of tester expression vectors, and

a target sequence encoding the target protein or peptide; and

either the first or second population of haploid yeast cells comprises a reporter construct comprising the reporter gene whose expression is under transcriptional control of the transcription activator.

7. (Amended) The method of claim 1, wherein the diversity of [the fusion proteins encoded by the library of yeast expression vectors] tester proteins in the library of tester fusion proteins is at least [1x10⁶] 1x10⁸.

8. (Amended) The method of claim 1, wherein the diversity of [the fusion proteins encoded by the library of yeast expression vectors] tester proteins in the library of tester fusion proteins is at least 1x10¹⁰.

9. (Amended) The method of claim 1, wherein the diversity of [the fusion proteins encoded by the library of yeast expression vectors] tester proteins in the library of tester fusion proteins is at least 1×10^{12} .

14. (Amended) The method of claim [16] 1, wherein the first [nucleotide sequence] polypeptide subunit in the library of [expression vectors] tester proteins comprises [a coding sequence of] an antibody heavy-chain variable region, and the second [nucleotide sequence] polypeptide subunit comprises [a coding sequence of] an antibody light-chain variable region.

18. (Amended) [The method of claim 1, further comprising:]

A method for selecting tester proteins capable of binding to a target peptide or protein, comprising:

(a) transforming a library of tester expression vectors into yeast cells which contain a reporter construct comprising a reporter gene whose expression is under transcriptional control of a transcription activator comprising an activation domain and a DNA binding domain, each tester expression vector comprising

a first transcription sequence encoding either the activation domain or the DNA binding domain of the transcription activator, and

a tester protein sequence comprising first nucleotide sequence encoding a first polypeptide subunit, a second nucleotide sequence encoding a second polypeptide subunit, and a linker sequence encoding a linker peptide that links the first and the second polypeptide subunits;

(b) transforming a target expression vector into the yeast cells simultaneously or sequentially with the library of tester expression vectors, the target expression vector comprising

a second transcription sequence encoding either the activation domain or the DNA binding domain of the transcription activator which is not expressed by the library of tester expression vectors; and

a target sequence encoding the target protein or peptide;

(c) expressing the tester fusion proteins from the library of tester expression vectors and the target fusion protein from the target expression vector;

(d) selecting those yeast clones in which the reporter gene is expressed, the expression of the reporter gene being activated by binding of the tester fusion protein to the target fusion protein;

(e) isolating the tester expression vector from the selected yeast clones; and

(f) mutagenizing the first and second nucleotide sequences in the isolated tester expression vectors to form a library of mutagenized expression vectors.

22. [The method of claim 1, wherein the target fusion protein comprises a human growth factor receptor]

A method for selecting single chain antibodies capable of binding to a human growth factor receptor, comprising:

(a) transforming a library of tester expression vectors into yeast cells which contain a reporter construct comprising the reporter gene whose expression is under transcriptional control of a transcription activator comprising an activation domain and a DNA binding domain, each tester expression vector comprising

a first transcription sequence encoding either the activation domain or the DNA binding domain of the transcription activator, and

a tester protein sequence comprising first nucleotide sequence encoding an antibody heavy chain variable region, a second nucleotide sequence encoding an antibody light chain variable region, and a linker sequence encoding a linker peptide that links the antibody heavy chain and light chain variable regions;

(b) transforming a target expression vector into the yeast cells simultaneously or sequentially with the library of tester expression vectors, the target expression vector comprising

a second transcription sequence encoding either the activation domain or the DNA binding domain of the transcription activator which is not expressed by the library of tester expression vectors, and

a target sequence encoding a human growth factor receptor;

(c) expressing the tester fusion proteins from the library of tester expression vectors and the target fusion protein from the target expression vector; and

(d) selecting those yeast clones in which the reporter gene is expressed, the expression of the reporter gene being activated by binding of the tester fusion protein to the target fusion protein.

B